STUDIES OF THE DEVELOPMENT OF THE CARTILAGE IN THE EMBRYO OF THE CHICK AND MAN.

M. L. HOLBROOK, M. D., New York, N. Y.

The first tissue formed in the embryo of the medullary tissue is cartilaginous. This fact is acknowledged by embryologists. The process of its development has remained unsettled for obvious reasons. So long as the minute structure of cartilaginous tissue was unknown, no correct idea of its manner of development was possible, and widely different views have been put forward by different observers.

Since the discovery that the basis-substance of hyaline cartilage is not a homogeneous mass but contains much living matter arranged in a reticular manner, the question as to how cartilage develops is very greatly simplified and all previous notions on this subject must be abandoned.

On the fifth day we find in the embryo of the chick all the organs of the thoracic and abdominal cavities distinctly recognizable. On the outer portion of the chest we find beautifully developed cartilage which on account of its regular arrangement must be considered the cartilaginous basis of the future ribs.

To prepare the embryo for cutting and examination we subject it first to a hardening process in a $\frac{1}{10}$ per cent. solution of chromic acid and gradually increase the acid up to about $\frac{1}{2}$ per cent. solution. It is then treated with alcohol for a short time, and now imbedded in celloidin. I used one of the section cutters of Toma of Heidelberg and found it quite satisfactory for obtaining extremely thin sections throughout the entire embryo. I mounted them in glycerine with the surrounding frame of celloidin. In studying the cartilaginous portions of these sections with a power of 500 or 600 diameters the basis-substance appears structureless, and in

most places represents about the width of one and sometimes two cartilage corpuscles. The latter lie very near each other. No trace of territories can be seen, such as are so plainly marked in the hyaline cartilage of grown and growing persons. Neither is there a difference in the aspect of the different portions of the basis-substance as is seen in the grown up animal and so often conveys the idea of a capsule surrounding each single corpuscle.

All the corpuscles in the embryo of the chick appear to be finely granular and most of them contain one central nucleus. Exactly the same features are seen in the embryo of the same age in transverse sections of the nota chord (*Chorda dorsalis*), the central portion of which has a cavity with medullary corpuscles. If we place the same specimens under a power of 1200 diameters the minute structure of the cartilage corpuscles becomes plainly visible. Wherever a nucleus is seen it exhibits a delicate reticular structure with several larger granules, the so-called nucleoli. The nucleus is also connected with a delicate reticulum throughout the body of the cartilage corpuscle, the points of intersection being generally smaller than those of the nucleus. Sometimes no nucleus is perceptible in the cartilage corpuscle, but a more or less uniformly arranged reticulum, with coarse and fine granules at the points of intersection, pervades its body. Sometimes one or more rows of granules surround the central larger granules, all being connected by threads of a substance of the same color and refraction as the granules themselves. Every single cartilage corpuscle under proper focus appears to be surrounded by a light, narrow rim, and this rim in many instances is traversed by delicate, radiating spokes which originate from the periphery of the cartilage corpuscle with a somewhat broader base. These fine ends point towards the slightly opaque basis-substance enclosing the light rims around the cartilage corpuscles.

The basis-substance itself everywhere appears to be endowed with a delicate reticulum, but its opacity slightly exceeds that of the grayish basis-substance. I emphasize the fact that these features appear in the specimens treated in the simple manner previously described and with the addition of no reagent whatever.

All that is seen is only suggestive of a structure within the basis-substance. To bring this into greater prominence I stained some sections with a half per cent. solution of chloride of gold, first carefully washing them in distilled water. Some of the sections were stained only thirty minutes, others an hour and others two hours. The last proved best. They were very carefully cleansed in distilled water to remove the surplus gold, and mounted in glycerine. Each corpuscle gave a dark violet hue. The basis-substance was

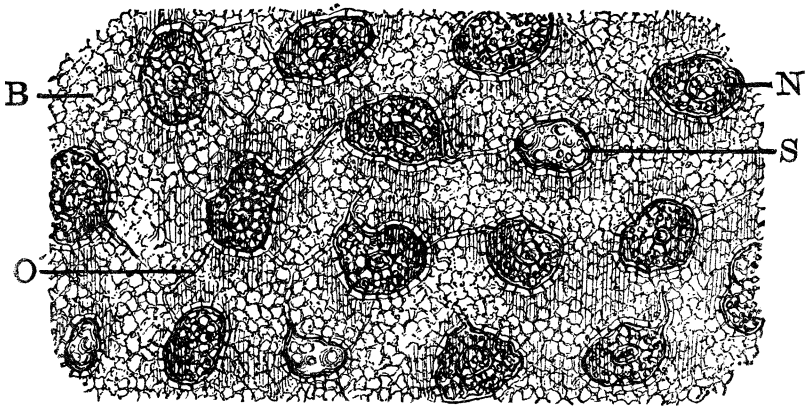

Fig. 1.—Cartilage of rib of chicken after five days' incubation, unstained. × 1200.

N.—Nucleated cartilage corpuscles.

S.—Solid, slightly vacuoled cartilage corpuscles.

B.—Basis-substance traversed by a delicate reticulum, the same as the nucleated cartilage corpuscles.

O.—Boundary line between previous medullary corpuscles that have been transformed into basis-substance.

paler but of a most beautiful violet pink and showing plainly the spokes of living matter emanating from the corpuscles themselves and penetrating the basis-substance.

Since we know that it is the living matter which becomes violet upon being treated with chloride of gold, it is fair to conclude that the cartilage corpuscles and basis-substance are traversed by living matter arranged reticularly; the difference between the two being that living matter in the cartilage corpuscle is more compact and coarse, that is, the basis-substance more delicate, more uniform and with slightly wider meshes which are filled up with the basis-substance proper.

I have traced the same features in the cartilaginous fields of the human embryo four weeks old, the chest of which was cut into horizontal sections. In a vertical section of the facial region of the human embryo six weeks old, the lower jaw cartilage was studied. This represented what was termed the primordial cartilage by Merkel, from which the jawbone is known to arise. The appearance of the cartilage was somewhat different from that of the embryo of four weeks, the cartilage corpuscles being nearly double the size and

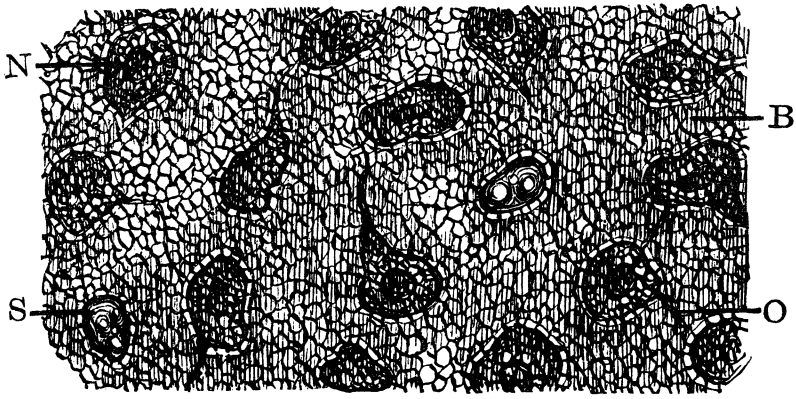

Fig. 2.—Cartilage of rib of chicken embryo after five days' incubation, deeply stained with chloride of gold. ×1200.

N.—Nucleated cartilage corpuscle.

S.—Solid cartilage corpuscle, slightly vacuoled.

B.—Basis-substance traversed by distinct reticulum, the same as the nucleated cartilage corpuscles.

O.—Boundary line between corpuscles that have been transformed into basis-substance.

standing apart at almost uniform distances. Where calcareous depositions had taken place in this specimen, the depositions were invariably seen first along the borders of the territories between the corpuscles. Even in advance of the deposition of lime salts, the first outlines of the territories were distinctly traceable by grayish lines. The calcareous deposits appeared in the shape of highly glistening granules, first in a single row and afterwards in broader trabiculæ, while the cartilage corpuscles are apparently enlarged and split up into medullary corpuscles, the well-known stage preceding ossification.

In the six-weeks human embryo the first traces of bone tissue are

seen at the borders of the primordial cartilage, obviously springing from it. The same stage of calcification of cartilage along the boundaries of the territories, with transformation of cartilaginous tissue into medullary tissue and the first stages of bone tissue springing therefrom, is easily traceable on the condyles of the shaft bones of a chicken six weeks old.

From what has been said in regard to the amount of basis-substance present in the cartilage of a chick embryo five days old and of a human embryo four weeks old it will be seen that the first formed cartilage is not identical with that of a somewhat older embryo. In the first case the distance between the single cartilage corpuscles being about the same as the diameters of the corpuscles, we may, I think, infer that here only single medullary corpuscles have taken part in the formation of basis-substance. We may understand the formation of such cartilage by assuming the transformation of single medullary corpuscles into basis-substance on the same plan on which the first medullary or myxomotous tissue is formed. Later, evidently after the first-formed cartilage had again returned into its medullary condition, a more perfect cartilage tissue is formed by the participation of several medullary corpuscles in the production of a single territory of cartilage tissue. This later cartilage, after the calcification of its basis-substance and its return to the medullary condition, yields bone tissue, although never in the way of direct transformation. In all cases the medullary corpuscles are transformed into basis-substances and do not perish at all, but the liquid contents of the meshes of their reticulum is transformed into the more nearly solid light-refracting substance, while the reticulum of the living matter itself is still present, but less visible, or is even invisible, except to a trained eye. When, however, the section has been stained with chloride of gold, this structure becomes conspicuous. The medullary corpuscles are connected with each other by the reticulum of living matter not only in their myxomotous condition, but after they have been connected into cartilage. Neither in the medullary nor in the cartilage tissue are there any isolated unconnected cells. It is the presence of living matter in the basis-substance of cartilage which enables us to understand the breaking down of both cartilage corpuscles and basis-substance into

medullary corpuscles, a sort of rejuvenescence which is admitted by good authorities and which leads at last to the formation of bone tissue.

The literature of this subject is abundant, but very few data are acceptable from our point of view. M. Foster and F. M. Balfour do not claim to treat in detail of histological changes which take place in the development of the embryo.* Albert Kölliker says: "The histological processes in the formation of cartilage are very simple. First, on all places that are to become cartilage the cellular elements increase in number and the parts under consideration become more dense and less transparent. Next, between the cells appears, at first scanty, later more abundant intercellular substance whenever the elements themselves become enlarged and transformed into light vesicles whereby the tissue becomes lighter and lighter and the cartilage is formed." †

On page 414 Kölliker indicates a somewhat different view and says: "From place to place a portion of the indifferent cells that form the external chorda sheath become differentiated by the formation of a homogeneous intercellular substance, and by increasing in amount it is changed into cartilage tissue. So long as the secretion theory was held by histologists, the process of the formation of cartilage was apparently very simple. The original medullary corpuscles produced a sort of secretion which became the basis or intercellular substance, and medullary corpuscles now became cartilage corpuscles."

Max Schultze in 1861 first pointed out the origin of basis-substance from previous medullary corpuscles. E. Brücke likewise in 1861 maintained that the peripheral portion of the cartilage corpuscle was transformed into basis-substance, while the central nucleated portion remained protoplasmic and took on the features of the cartilage cell proper. As early as 1867 C. Fromman recognized some structure in the basis-substance which he describes as follows: "The intercellular substance of the cartilage exhibited a finely granular structure, looked in some places as if dusty, in others be-

* Elements of Embryology: London, 1883, page 270.

† Entwickelungs Geschichte des Menschen: Leipsic, 1879, page 439.

sides the granules were fibers of great delicacy. These fibers were short and run in all directions." *

This description, though imperfect, plainly shows that to Fromman is due the first suggestion of a reticular structure in the basis-substance.

Later, C. Heitzman,† by means of reagents, mainly the nitrate of silver and chloride of gold, also by the study of the process of the calcification of the cartilage in normal and pathological conditions, came to the conclusion that the basis-substance of cartilage was endowed with a reticular structure, in all essential points identical with that of the cartilage corpuscles themselves. The reticulum is claimed to be the living matter proper, thus maintaining the properties of life for the basis-substance as well as for the cartilage corpuscles. He corroborated the views of Max Schultze with regard to the formation of basis-substance in general and that of cartilage in particular. According to him, the medullary corpuscles, upon being transformed into basis-substance, do not perish, but only the liquid portion held in the meshes of the reticulum becomes transformed into a comparatively solid and the light, highly refractory basis-substance, while the reticulum remains unchanged, simply becoming invisible.

That the basis-substance is endowed with properties of life has been, later, proven by Arnold Spina and by Stricher of Vienna, and Louis Elsburg of New York.

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* 'Untersuchen uber die Normale und Pathologische Anat., des Ruckenmarks, Zweite Theil, page 30.

†Untersuchungen uber das Protoplasma, Sitzungs Berichte Wiener Akad. d Wissensch., 1872.